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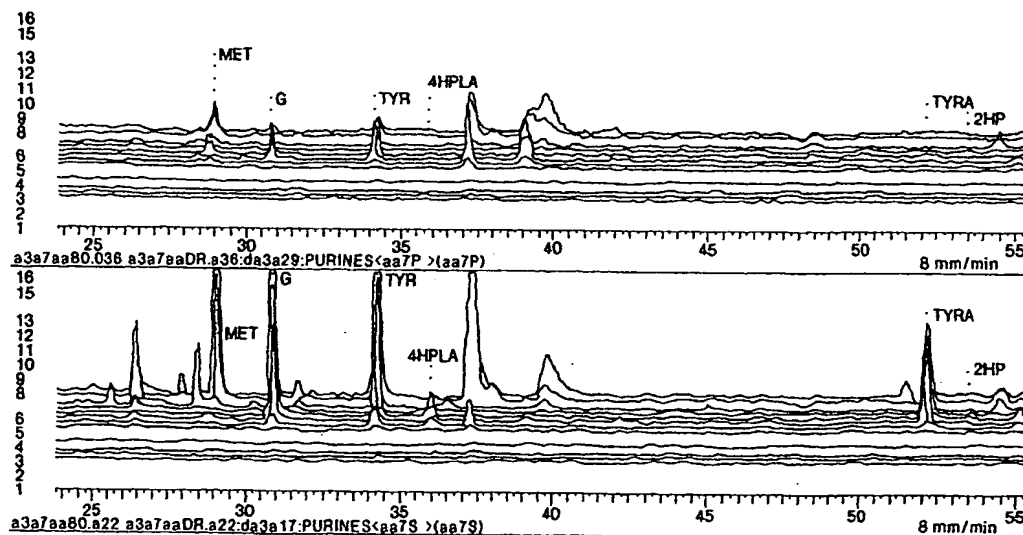
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(54) Title: DETECTION OF DNA DAMAGE



Comparison of supernate and cell patterns from PAP smear sample.  
Note: This illustrates one of the criteria for initially selecting tyramine (TYRA) as a normalizer.

(57) Abstract: The health condition of a living organism is detected by electrochemically analyzing samples from selected areas of the body of said living organism for elevated free levels of nucleotide excision products resulting from DNA or RNA damage.

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## DETECTION OF DNA DAMAGE

### Field of the Invention

The present invention relates to improvements in diagnostic techniques, and more particularly to diagnostic techniques for detecting and identifying DNA or RNA damage. The invention has particular utility in the detection of cervical cancer and will be described in connection with such utility, although other utilities are contemplated, as will be discussed below in detail, including detection of other cancers and other disease conditions, as well as health conditions brought out by exposure to environmental insults such as radiation, metals, smoke and solvents.

### Background of the Invention

The current gold standard for detecting cervical cancer in women is the so-called "PAP Smear." However, the reliability of PAP Smear testing, which relies upon a technician's observations, under the microscope, of cellular morphology, may be compromised by technician fatigue and/or subjectivity. Even very experienced technicians may misread a slide. If a false-positive is "called" or the slide results in an "uncertain" call, the physician may err on the side of the patient's safety, and call for a hysterectomy (uterus removal) or total hysterectomy (uterus and ovary removal). This results in the patient taking a lifetime dose of hormone replacement medications to keep the body in balance. Of course, the failure to identify "a pre-cancerous condition" could lead to an even more disastrous result to a patient.

### Object of the Invention

It is thus a primary object of the invention to overcome the aforesaid and other disadvantages of the prior art. Another object of the invention is to provide an analytical technique for detecting and diagnosing disease conditions, as well as health conditions due to exposure to environmental conditions, by detecting and identifying DNA or RNA damage markers. Another more object of the invention is to provide a reliable, totally objective analytical technique for detecting cancer.

### Brief Description of the Invention

1 In order to effect the foregoing and other objects, the present invention  
2 provides an analytical technique for detecting cancer or other disease and/or  
3 health conditions based on measurement of free levels of nucleotide excision  
4 products resulting from DNA or RNA damage, such as 8OH2'dG-8 hydroxy  
5 2' deoxyguanosine; O6MG - O-6-methylguanine; 2'dG - 2'deoxyguanosine;  
6 7MG - 7-methylguanine; 8NG - 8-nitroguanine; 8OHG - 8-hydroxyguanine;  
7 8OH2'dA - 8-hydroxy-2-deoxyadenosine; 8OHA - 8-hydroxyadenine;  
8 5OH2'dCy - 5-hydroxy 2'deoxyctidine; 5OHU - 5 - hydroxyuracil; 3NT - 3-  
9 nitrotyrosine; or 3 CIT - 3-chlorotyrosine in biological samples from selected  
10 areas of the body. More particularly, the present invention is based on the  
11 hypothesis that specific areas of the body are semi-isolated in-situ biochemical  
12 environments for nucleotide excision products such as 8OH2'dG or other  
13 nucleotide excision products, and that the levels of such free 8OH2'dG or  
14 other nucleotide excision products in the semi-isolated environment magnify  
15 the combined defects of DNA or RNA damage and repair mechanisms. By  
16 way of specific example, the effective damage and repair rate increases in  
17 DNA in cancer, or pre-cancerous cells, has been found to be magnified by  
18 accumulation in the extra-cellular matrix in selected areas of the body of  
19 8OH2'dG. For example, as applied to cervical cancer, the cervix has been  
20 found to be a semi-isolated in-situ biochemical environment which may be  
21 accessed through cervico vaginal lavage sampling. Thus, an objective  
22 analytical technique for determination of cervical cancer is provided.

### Brief Description of the Drawings

24 For a further understand of nature and objects of the present invention,  
25 presence should be had to the following detailed description taken in  
26 conjunction with the accompanying drawings wherein:  
27

1        Fig. 1 is a schematic view of one form of sample separation and  
2        analysis system in accordance with the preferred embodiment of the  
3        invention;

4        Fig. 2 is a side elevational view, in cross section, showing details of a  
5        preferred form of sample preparation column useful in accordance with a  
6        preferred embodiment of the present invention;

7        Fig. 3 is a side elevational view, in cross section, of a separation and/or  
8        testing column controlled as an electrochemical cell useful in accordance with  
9        a preferred embodiment of the invention;

10       Figs. 4 and 5 are a series of graphs showing the current over time of an  
11       electrochemical analysis for 8OH2'dG and O6MG, respectively, in cervico  
12       vaginal lavage samples in accordance with the present invention; and

13       Fig. 6 are coulometric electrode array system patterns of PAP smear  
14       samples in accordance with the present invention.

15       Detailed Description of the Preferred Embodiment of the Invention

16       Further understanding of the features and advantages of the present  
17       invention will be had from the following detailed description of the invention,  
18       which illustrates the electrochemical analysis of 8 hydroxy 2' deoxyguanosine  
19       (8OH2'dG) in cervico vaginal lavage or PAP smear swab samples. Analysis  
20       is accomplished using an electrochemical analytical system made in  
21       accordance with and following the general analytical procedures disclosed in  
22       PCT Application No. PCT/US98/22275, and as discussed in "A Carbon  
23       Column Based LCEC Approach to Routine 8-Hydroxy-2'-Doxyguanosine  
24       Measurements in Urine and Other Biological Matrices" (Bogdanov MB, et al.  
25       Free Rad. Biol. Med. 27, 1227-1248, 1999).

26       The conventional hypothesis of hydroxyl radical DNA damage and  
27       repair and excretion of the hydroxylated adduct 8OH2'dG is that a hydroxyl  
28       radical reacts with the DNA causing hydroxylation of the deoxyguanosine at  
29       the 8 position. The damaged segment is then either excised by glycolysis as

1 the 8Ohgua or by endonuclease excision as the 8OH2'dG5' monophosphate.  
2 The monophosphate is then dephosphorylated and the 8OH2'dG is rapidly  
3 excreted from the cell. The 8OH2'dG excreted to the extracellular matrix is  
4 then cleared rapidly from the body primarily via the kidney and excretion in  
5 the urine. 8Ohgua can also be produced as a result of attack on RNA and  
6 glycolysis.

7 Studies performed with the technologies described above have  
8 confirmed certain basic elements of this hypothesis as follows:

9 1. Dialysis studies of 8OH2'dG in extracellular matrix with probes  
10 placed in brain and muscle of rat and mouse to measure the rate of  
11 production and excretion compared with the free levels inside the cellular  
12 material confirm that excretion from the cells is rapid. Similarly, comparison  
13 of CSF, Plasma and urinary levels of ca 1:10:2000 in ca 100 ALS and control  
14 subjects confirm that the rate of clearance from the body is high. Studies of  
15 saliva or sweat vs. urine levels and studies showing highly elevated levels in  
16 plasma kidney dialysis patients confirm that urinary excretion is the primary  
17 mode of removal.

18 2. Studies of urinary levels of 8OH2'dG within individuals over  
19 diurnal weekly, monthly and yearly intervals indicate that the rate of DNA  
20 damage and repair is highly constant and characteristic of an individual.  
21 Similarly, studies of siblings and parents which show a significantly closer  
22 agreement of sibling values indicate that the rate of DNA damage is strongly  
23 determined by genetic factors.

24 Other studies, however, have indicated some basic difficulties with the  
25 simplistic model of damage and repair which have relevance to the use of  
26 DNA damage markers as diagnostic tools.

27 1. The simple model of increased production of hydroxyl radical  
28 leading to increased DNA damage is incomplete.

1           Comparison of total body rate of production of hydroxyl radical  
2   estimated by salicylate spin trappings showed no correlation in normal  
3   individuals, ALS patients and Freidrich's Ataxia FA patients of urine or  
4   plasma levels of 8OH2'dG. Nor was there any group elevation of total body  
5   hydroxyl radical production in FA or ALS although the 8OH2'dG levels were  
6   increased in CSF plasma or urine by 25-30%.

7           Urine levels of 8OH2'dG were significantly increased in individuals  
8   exposed to arsenic or o-toluidine or aniline although these materials play no  
9   direct role in the increase of hydroxyl radical production.

10          These studies suggest that conformational changes in DNA induced by  
11   exogenous or indogenous adducts or changes in sub cellular structures in  
12   proximity to DNA play a stronger role in increasing the rate of DNA damage  
13   than overall hydroxyl radial production. Also supporting this concept are  
14   studies I have performed showing no effect of simple antioxidants such as  
15   Ascorbate or Tocopherol on the levels of 8OH2'dG.

16          2.       Relevant to the use of DNA damage rate measurements to  
17   cancer diagnosis I have observed that in C. Elegans culture there is a burst of  
18   8OH2'dG production during the stage of rapid replication of the gonadal  
19   cells. In similar experiments I observed that a toxin (3 nitro propionic acid,  
20   3NP) increased the rate of DNA damage during replication. When the toxin  
21   was removed after exposure the rate of damage was still maintained at a  
22   higher level indicating that the initiation of a higher rate of damage creates a  
23   biochemical state or feed back that persists beyond the time of the applied  
24   insult.

25          3.       It has also been observed in human controls, ALS patients and  
26   FA patients and in C. Elegans culture that the levels of 8OH2'dG that remain  
27   in the DNA (measured by extracting and hydrolyzing the DNA) are only  
28   weakly correlated with the rate of output of the 8OH2'dG. This indicates that  
29   both damage and repair are up regulated simultaneously and thus that

1 measures of the free levels of excised DNA damage product provide a much  
2 more robust indicator of disorder related DNA damage processes.

3         4. In situations where there is a high rate of damage and repair the  
4 conventional sequence of excision of the 8OH2'dG phosphate,  
5 dephosphorylation of the 8OH2'dG and excretion of the 8OH2'dG does not  
6 hold. In rapid cell replication in C. Elegans, in 3NP insulted C. Elegans and in  
7 Cervix cancer cells (as described below) the 8OH2'dG phosphates are directly  
8 excreted.

9         5. Sampling specific to the site of insult is of considerable  
10 importance. In studies of smoking related increases of urinary 8OH2'dG  
11 levels, I have found a statistically significant increase of only 11% in a cohort  
12 of ca 200 smokers vs. ca 300 control non-smokers. However, the free levels of  
13 8OH2'dG in the extra-cellular matrix from pharyngeal swabs of smokers vs.  
14 non-smokers are elevated by a factor of 3x-4x when normalized against the  
15 cellular metabolite of tyrosine, 4-hydroxy phenyl lactic acid.

16         The synthesis of these studies and observations leading to the  
17 approach to cancer diagnosis is as follows:

18         1. When a cell begins extensive abnormal replication the changes  
19 in the conformation of the DNA during mitosis make it more susceptible to  
20 free radical damage and both increase the levels of excised damage products  
21 and change the nature of those products.

22         2. Sample sites which are semi-isolated in situ from physiological  
23 transport and excretion, reflecting the extracellular matrix around the affected  
24 cells will show highly increased levels of the repair products relative to the  
25 damage products remaining.

26         3. Levels of the repair products can be normalized against other  
27 cellular metabolites that are excreted to the extracellular matrix from  
28 metabolic processes that are not materially affected by free radical damage.  
29

Working Examples

Cervico vaginal lavage samples were centrifuged on acquisition to separate the exfoliated cells and the supernatant containing the extracellular matrix. PAP smear swabs were vortexed in a normal saline solution and centrifuged to separate the exfoliated cells and extracellular matrix.

Analysis for 8OH2'dG in supernatants was accomplished in accordance with the teachings of PCT/US98/22275 using electrode preparation and sample concentration protocol described in Bogdanor et al, supra. Analysis for 8OH2'dG phosphates was accomplished by treating a sub aliquot of the supernatants with alkaline phosphatase following the last stage of the DNA hydrolysis protocol described in Bogdanor et al, and determination of the 8OH2'dG phosphate level by difference between the alkaline phosphatase treated and the non-treated aliquot. Chlorotyrosine measurements of subaliquots of the supernatant were made according to the teachings in PCT/US98/22275 following the protocols in Bogdanor et al.

The process for determining appropriate normalizers and markers of sample integrity was to prepare and analyze extracts of the supernate and cells from cervico vaginal lavage and PAP smear samples, plasma red blood cell and leucocyte samples, buccal cell samples and induced sputum samples following the procedures in Kristal et al "Simultaneous Analysis of the Majority of Low-Molecular Weight Redox-Active Compounds from Mitochondria," Anal. Biochem. 263, 18-25(1998). Typically 600 peaks of redox active compounds are isolated from such preparations. The various samples were compared against themselves and against authentic reference standard mixtures primarily to search for peaks or unknown compounds with the following characteristics:

1. The peaks should be unique to cervico vaginal lavage or PAP smear samples in order that it not be compromised by inclusion of other cellular or biological material inclusion.



1           2.       The peaks should be present in the supernate and not in the  
2 cellular extracts or at significantly higher relative concentration than the  
3 cellular extract in order to reflect a similar high rate of excretion from the cells  
4 as 8OH2'dG.

5           Secondarily, the patterns of cervico vaginal lavage an PAP smear  
6 samples were compared against urine and blood samples to determine the  
7 level for compounds that are highly elevated in blood, e.g. glutathione and  
8 urine, e.g. uric acid such that levels of these compounds indicate  
9 contamination and effects on 8OH2'dG levels could be established.

10          Of 16 peaks in the patterns meeting the criteria 1 and 2 Tyramine was  
11 identified. The other 15 peaks meeting the criteria are currently of unknown  
12 structure although they are also candidates for normalization. Levels of uric  
13 acid and glutathione in cellular material and supernate from cervico vaginal  
14 lavage and PAP smear samples established levels of ca 1000 ng/ml or uric  
15 acid and 50,000 ng/ml of glutathione below which no measurable impact on  
16 the 8OH2'dG would be expected. A typical example of the comparison for  
17 known compounds among cell types is shown in Table 1.

18          The carbon column switching instrumentation and the array  
19 instrumentation were combined by placing an 8 channel series sensor on the  
20 output of the first analytical column of the carbon column switching  
21 apparatus and a second 8 channel series sensor on the output of the second  
22 analytical column of the carbon column switching apparatus. This allowed  
23 the simultaneous determination of the uric acid glutathione and tyramine on  
24 the output of the first analytical column and the carbon column trapping,  
25 elimination of interferences and detection of 8OH2'dG and Chlorotyrosine on  
26 the second analytical column.

27          Figure 4 and 5 show typical responses for 8OH2'dG and O6MG in PAP  
28 smear samples. The figures illustrate the detection of 8OH2'dG in all samples  
29 and the limitation in detecting O6MG in some.

1           Figure 6 illustrates the CEAS patterns observed from cellular and  
2           supernatant preparations. These were compared against each other and  
3           against similar patterns of urine, plasma, whole blood and buccal cells to  
4           determine normalizing compounds and indicators of sample quality.

5           The results of the analytical protocols and concepts applied to 51  
6           cervico vaginal lavage samples from 51 patients with various diagnoses and  
7           two normal PAP smear samples are shown in Table I.

8           Table II presents the data on 8OH2'dG, O6MG vs. an abbreviated  
9           diagnostic categorization ranked by tyramine divisor and sample weight or  
10          weight estimated from protein. CVL and PAP smear samples are ranked  
11          separately. Initial studies of vaginal vs. cervical swabs indicated higher levels  
12          of tyramine in the former. It is thus likely that tyramine is not an optimum  
13          normalizer since there are several other peaks that meet the criteria but have  
14          not been chemically identified. Table III presents preliminary data on  
15          relationships among free and DNA levels of 8OH2'dG, in the cervix and urine  
16          and plasma. There are some limitations on the data quality affecting  
17          interpretation. Precision on small samples (less than 2 mg) is only +/-220-  
18          30%. Storage histories are not completely documented and there are  
19          uncertainties associated with any study of a new matrix. However, some  
20          observations are indicated.

21

1

TABLE I (Part One)

CASE						
Compound 00000001 ora 00000003 ora 00000006 ora 00000008 ora 00000009 ora						
ng/ml	CL	BUC	MUC	BUC	MUC	CL
2HPAC		0.63		0.40	0.20	0.09
3MT	0.13	0.19		0.18	0.08	0.12
3OHKY	0.20	0.31		0.16	0.24	0.15
3OMD	0.76	0.31		0.39	0.44	0.32
4HBAC	0.26	0.44			3.26	2.94
4HPAC	38.33	43.35		8.45	8.27	9.35
4HPLA	120.24	92.50		31.85	28.88	24.37
5HIAA	0.47	0.22		0.07	0.24	0.08
5HT	0.10			0.11	0.11	1.13
5HTP	0.06	0.11		0.16	0.19	
AM	6.48	0.08		0.07	0.10	0.06
ASC	0.62	0.13			1011.01	
CYS	70.29	72.53		54.63	82.85	70.29
DA	21.93	6.66		0.94	3.27	14.19
DOPAC	0.11	0.11		0.12	0.09	0.13
G	12.52	8.62		3.44	237.98	19.46
GR	12.47	32.41		6.45	30.36	3.53
GSSG	3.18	111.76		25.78	24.08	42.78
HGA	0.19	0.11		0.25		0.14
HVA	0.23	0.16		0.09	0.10	0.07
HX	24.75	39.98		8.10	619.81	48.07
KYN	1.03	0.09		24.39	1.63	0.29
LD	1.23	2.43		0.59	0.57	1.29
MEL	0.92	0.92		1.10	4.49	4.81
MET	42.88	0.82		4.21	441.49	43.94
MHPG	0.46	0.51		0.55	0.42	0.44
MN	1.22	0.17		0.65	0.38	0.28
NASHT	0.14	0.17		0.11	0.14	0.21
NE	0.40	0.18		0.33	0.19	0.36
NMN	0.30	0.51		0.28	0.32	0.49
TPOL	8.59	6.11		7.77	9.11	11.33
TRP	186.72	27.57		7.44	240.92	47.34
TRYPT	0.15	1.19		0.61	0.83	82.32
TYR	266.09	551.51		251.17	717.64	98.95
TYRA	579.81	10.61		0.19	0.90	525.82
URIC	695.51	703.83		3209.99	221.60	48.09
VMA	0.44	0.43		0.12	0.26	
XAN	73.44	235.05		16.67	107.60	16.00
XANTHOSINE	19.29	83.74		1.75	37.00	20.15
NORMALIZERS						
8OHdG pg/ml	11.2	3.33		2.22	8.47	10.8
80h/tyrx100	4.21	0.60		0.88	1.18	10.91
8oh/cysx100	15.93	4.59		4.06	10.22	15.36
8oh/tyrax1000	19.32	313.85		11684.21	9411.11	20.54

2  
3

1

TABLE I (Part Two)

CASE 00000014.ora 00000016.ora 00000017.ora 00000024.ora 00000029.ora 00000033.ora

Compound	BLOOD.ca1:15 CL						
ng/ml	BUC	MUC	CL	CL	CL		
2HPAC	0.25		0.62	0.24	294.78	3.23	1.96
3MT	0.17		0.11	0.22	1.39		0.33
3OHKY	0.16		0.48		0.65		0.52
3OMD	0.39		0.51		0.53	1.31	0.39
4HBAC	3.85		6.78	0.47	1.83	0.72	0.19
4HPAC	1.49	244.44		4.48	658.94	0.99	24.89
4HPLA	4.67	66.41	37.64		665.94	0.75	55.39
5HIAA	0.10		0.48			0.15	0.19
5HT	0.11		0.26			0.79	0.19
5HTP	0.06		0.24	0.54	0.16		0.13
AM	0.25		1.70	0.11	13.69	0.11	0.40
ASC	0.35		0.16	416.21	5.77	1.14	0.62
CYS	76.22	88.99	45.98	49.89		21.61	48.44
DA	1.25	2.01	0.38	3.33		0.43	0.41
DOPAC	0.25	0.18		1.40		0.37	0.22
G	3.63	61.18	89.06	1.38		1.27	86.94
GR	11.75	59.25	5.07	16.22		11.61	130.11
GSSG	37.48	193.42	27.37	2.12	1901.78		130.07
HGA	0.16		0.22	0.25		0.51	0.23
HVA	0.14	0.15	0.18	0.10		0.38	0.57
HX	74.60	461.74	152.57	2441.70	2445.10		112.92
KYN	0.07	5.09	0.48	28.95		0.38	9.74
LD	0.50	0.50	0.65	3.26		0.67	0.91
MEL	0.16	1.81	0.22	0.62		0.49	1.02
MET	1.27	464.70	39.17	73.65	831.72		867.84
MHPG	0.48	0.61		0.95		0.66	0.40
MN	0.20	0.39	0.26	0.25		0.57	1.28
NASHT	0.17	0.17		0.18			0.12
NE	0.26	0.32	0.26				0.22
NMN	0.29	0.50	0.37	0.34		0.32	3.30
TPOL	13.89	16.47	36.25	1.19		1.41	28.63
TRP	5.07	53.65	55.25	3904.06	523.63		237.29
TRYPT	0.43	8.19	0.74	13.85		0.50	277.80
TYR	140.30	214.95	227.17	7411.08	1545.97		639.34
TYRA	2.03	772.19	330.77	35.39		0.15	1088.90
URIC	580.64	229.01	405.36	314.90	1992.77		371.60
VMA	0.10	0.27	0.50	0.17			1.12
XAN	53.93	1458.70	58.20	3669.12	343.39		45.54
XANTHOSINE	21.78	23.70	16.64	1461.96	151.37		34.65
NORMALIZERS							
8OHdG pg/ml	4.9	17.39	2.42	635.48	0.32		1.61
8oh/lyrx100	3.49	8.09	1.07	8.57	0.02		0.25
8oh/cysx100	6.43	19.54	5.26	1273.76	1.48		3.32
8oh/lyrax1000	2413.79	22.52	7.32	17956.49	2133.33		1.48

2

3

TABLE II

CASE CODE	8OH*1000/lyr	DIAG CODE	8OH pg/gm	8OH/06MG	CASE CODE	8OH*1000/lyr	DIAG CODE	8OH pg/gm	8OH/06MG
CVL SAMPLES					PAP SMEAR SAMPLES				
ESA006	17552.22	C	108934	0.12	CON1R1	0.92 N		368	7.2
ESA041	5198.99	C	67132		CON2	1.03 N		442	11.2
ESA0447/21	3827.08	C	38897		CON3	0.83 N		293	14.1
ESA0447/16	3452.49	C	42132	0.24	CON4R1	0.72 N		528	NA
ESA051	2441.30	C3	44150		CON1R5				
ESA037	1881.84	C3	31988		endo brush	1.06 N		445	6.6
ESA011	1600.00	G	NA		exo brush	0.94 N		502	NA
ESA048	1373.63	A,D,R	12012		vaginal padl	0.12 N		28	NA
ESA033R3	940.82	A,D,R	18821	1.33	CON5R1	2.44 N		306	11.1
ESA025CVL	835.73	A,R	28812		CON4R3	0.98 N		122	4.3
ESA033R1	564.89	A,R	15166		CON5R3	1.87 N		354	7.5
ESA034	513.59	A,D,R	12997	0.92					
ESA030	513.55	A,R	19281		W001	2.19 A,R		125	1.25
ESA043	510.66	A,R	12113	0.77	W002	4.1 A,R		507	0.73
ESA038	502.68	A,R	23881		W003	58.26 Hepatitis		3855	0.57
ESA040	215.30	A,R	NA		W004	8.29 A,R		200	0.56
ESA050	197.87	A,R	10667	1.92	W005	2.19 N		75	1.58
esa032s	121.11	A,D,R	12188		W006	4.3 N,R		500	0.84
esa029s	74.25	A,R	2108		W007	2.1 N		90	0.71
ESA035	38.50	A,D,R	1722		W008	27.49 N,HPV		361	0.55
ESA018CVL	35.74	A,R	3221		W009	113.04 ?A,HPV		1300	0.45
ESA022CVL	27.73	A,R	3324	1.01	W010	0.9 ?A		75	1.68
ESA004	22.53	A,R	NA		W011	1.6 N,R		75	1.2
ESA019CVL	22.05	A,D,R	1922		W012	1.8 N		86	1.48
ESA003	20.53	A,R	1334		W013	1.1 N		75	1.2
ESA023CVL	20.49	A,R	1987		W014	1.8 N,R		145	1.8
ESA001	19.31	A,D,R	1332		W015	1.9 N		199	4.4
ESA039	15.53	A,R	1435		W016	2.6 N,R		301	4.3
ESA005	7.33	A	397		W017	2.8 A,R		190	1.3
ESA036	3.79	A	433		W018	3.12 A		412	1.9
esa027CVL	2.91	A	198		W019	1.4 N		138	7.3
ESA047	1.56	N	163 GT10		W020	1.87 A		115	4.4
ESA012	1.48	N	289		W021	4.12 N,R		543	1.7
ESA049	1.33	N	143		W022	1.7 A		214	GT10
ESA020CVL	0.71	A	206		W023	56.9 N,HPV		3224	0.41
esa031s	0.71	N	134		W024	2.1 A,R		341	3
ESA046	0.64	N	289		W025	0.81 N		104	NA
ESA021CVL	0.61	A	189	7.44	W026	2.3 N,R		122	5.7
ESA042	0.57	N	215		W027	2.8 A,R		107	4.4
ESA045	0.45	N	127 GT10		W028	1.6 N		115	8.1
ESA026CVL	0.36	N	131						
ESA024CVL	0.18	N	156						

N-normal PAP, A-ASCUS, C-cancer, C3-CIN3, D-dysplasia, R-various risk factors, HPV-PCR+ viral test NA-not analysed,interference

1

TABLE III

URINE	CVL SAMPLE	URINE ug/gm	DNA pm/um	PLASMA
urine code	8OH <sup>+</sup> 1000/lyra	8OH <sup>2</sup> 'dG/creat	8OH <sup>2</sup> 'dG/2'dG	pg/ml 8OH <sup>2</sup> 'DG
esa001u		3.15		
esa003u		4.97		
esa004u		4.57		
esa005u	17652.22	14.48	5.81	31.90
esa012u	5198.99	3.30		16.30
esa006u	2441.30	5.73		
esa018u	1881.84	2.38	6.13	22.10
esa019u	1373.63	1.71		
esa020u	940.82	4.11		
	835.73	2.86	4.98	14.60
esa022u	564.89	3.94	5.87	
esa023u	513.59	1.79		
esa024u	513.55	4.83		
esa025u	510.66	2.19		
esa026u	502.68	2.22		
esa027u	215.30	5.38		
esa029u	197.87	4.14		
esa030u	121.11	2.16		
esa031u	74.25	1.64		11.80
esa032u	38.50	3.25		
esa033au	35.74	3.05		
esa033bu	27.73	2.40	3.44	
esa034u	22.53	2.15	3.38	15.30
esa035u	22.05	3.04		
esa036u	20.53	5.53	5.12	16.40
esa037	20.49	5.45	4.76	
esa038u	19.31	3.95		
esa039u	15.53	3.55		
esa040u	7.33	4.24	4.16	11.50
esa041u	3.79	2.13	4.28	
esa042u	2.91	4.44		
esa043u	1.56	3.64		
esa0447/16u	1.48	3.33	3.36	
esa0447/21u	1.33	3.71		
esa045u	0.71	3.24		12.30
esa046u	0.71	6.69		17.80
esa047u	0.64	1.81		
esa048u	0.61		2.58	
esa049u	0.57	3.29		
esa050u	0.45	2.50		
esa051u	0.36	4.11		
esa011u	0.18	2.50		

2

3

## RESULTS

Free levels of 8OH2'dG in pg/ml were 1.61 (normal case), 2.42 (precursor cell case) 10.8-17.4 (dysplasia cases n=3) and 635.5 (cervical cancer case). The values corrected for dilution by various measures of total protein, amino acids or cell count, indicate initial concentrations in the extracellular cervical environment of ca 80-110,000 pg/ml or ~1000 times the normal plasma levels of 10 pg/ml. This supports the hypothesis that the cervix is in a biochemically isolated area. Ratios of 8OH2'dG/2'dG in the DNA of exfoliated cells were only elevated by factors of 2.1 and 4.8 in the Dysplasia and cancer cases respectively vs. the normal. This also supports the hypothesis that the effect of increase in both DNA damage and repair is magnified in the extracellular matrix in the cervix environment.

It is also consistent with the intuitively less probable hypothesis that the DNA damage and repair is proceeding at a 20-100 fold higher rate in the cervix. A weak correlation ( $r=0.431$ ), driven by high values in the cancer patients between urinary and cervical levels, is also consistent. In the cases with repeat cervical samples, the values were consistent within an individual. Urinary 8OH2'dG values are highly individually specific and the same may hold for the cervix.

The absolute levels of 8OH2'dG based on estimates of the amount of extracellular matrix from protein values in cervico vaginal lavage samples and actual weights of PAP smear samples are on the order of 200-800 pg/ml or ca 20-80 times the levels of 10 pg/ml observed in plasma (2). This supports the hypothesis and necessary diagnostic requirement that the cervix is a semi-isolated in-situ area with respect to transport and excretion of DNA damage products.

The very slight not statistically significant increase in DNA damage products in the DNA itself supports the hypothesis that both damage and repair are up regulated and that the effect is magnified in the extracellular matrix.

1           While uncorrected 8OH2'dG concentrations are statistically higher in  
2   the cancer patients and patients at risk than in controls the use of Tyramine as  
3   a normalizer reflecting sample dilution and acquisition factors of the  
4   extracellular matrix is effective in compressing the scatter of the data.

5           Tyramine normalized levels of 8OH2'dG in all control samples and in  
6   two cases of findings of Abnormal Squamous Cells of Uncertain Significance  
7   (ASCUS) are all below the level of 1.6 pg 8OH2'dG/ng Tyramine. All subjects  
8   with identified cervix cancer and two with a high degree of suspicion of  
9   cancer are about 16000 pg 8OH2'dG/ng Tyramine. Patients with  
10   intermediate possibly precancerous problems and risk factors have  
11   8OH2'dG/Tyramine ratios that are intermediate between control and cancer  
12   and may predict an enhanced degree of risk of an early cancer that will  
13   manifest subsequently.

14           The presence of measurable 8OH2'dG phosphate as a significant  
15   portion of the total 8OH2'dG occurs in all the cancer cases and in three of the  
16   cases with significant risk factors and initial per cancerous indicators.

17           Chlorotyrosine is found in seven cases and may in the case of ESA051  
18   indicate a significant level of infection as well as or instead of an early cancer  
19   lesion.

20           Thus, the invention and concept provides an index of  
21   8OH2'dG/Tyramine that separates cervical cancer subjects from controls by a  
22   minimum factor of 1000 and gives intermediate values for various degrees of  
23   risk and findings of abnormal or precancerous cells or lesions. A unique  
24   marker of excretion of a portion of 8OH2'dG as the phosphate is found in all  
25   cancer subjects and may provide the first indicator of the culmination of risk  
26   and insult factors in an early cancer in the individuals with high  
27   8OH2'dG/Tyramine ratios. The ability to commensurate markers of  
28   inflammation and infection provides additional diagnostic discrimination  
29   between risk factors and actual development of a cancerous lesion.



1        Several possible candidates for dilution normalizing compounds also  
2        were identified from application of a protocol for analysis of the majority of  
3        oxidizable compounds in the cervical lavage supernate. Tyramine (TYRA)  
4        notably appears to be an attractive normalizer: -- it is a uniquely elevated  
5        product of cervical cellular metabolism relative to other cellular material  
6        (Buccal cells, lymphocytes, alveolar and pharyngeal lavage, brain and muscle  
7        dialysates); it is excreted rapidly to the extracellular matrix; and it is co-  
8        determinable with 8OH2'dG with the technology employed. For the six test  
9        cases, the mole ratios of 8OH2'dGx1000/TYRA were: 1.48 (normal case), 7.34  
10       (precursor cell case), 19.3-22.5 (Dysplasia cases n=3) and 17956 (Cervical  
11       cancer case).

12       Thus, there appears to be a correlative decrease in 8OH2'dG levels with  
13       increased coQ10 levels in plasma, or in the local in situ cervical area.  
14       Accordingly, the analytical strategy of measuring both free and DNA levels of  
15       8OH2'dG in conjunction with the coQ10 also provides a basis for  
16       discriminating between changes in the rate of oxidative damage and changes  
17       in the rate of repair associated with the coQ10 levels.

18       The invention is susceptible to modification. For example, analysis of  
19       stool samples for the presence and level of 8OH2'dG and 8OH2'dG  
20       phosphates (which typically do not occur above about 0.1pg/gm in normal  
21       feces) both as timed or total output and normalized against levels of the  
22       tyrosine metabolite gentisic acid may be employed as a diagnostic of colon or  
23       lower bowel cancer; analysis of saliva for elevated levels of 8OH2'dG may be  
24       employed to provide an early test for esophageal or throat cancer; analysis of  
25       urine or semen for elevated levels of 8OH2'dG may be employed to provide  
26       an early test for bladder or testicular cancer; pharyngeal or nasal swab,  
27       alveolar or bronchial lavage samples for analysis of 8OH2'dG normalized  
28       against levels of 4-Hydroxy Phenyl lactic acid may be employed as a  
29       diagnostic of pharyngeal, nasal, bronchial or lung cancer respectively;

1 analysis of foliated urithelial cells in timed culture for 8OH2'dG levels  
2 normalized against cell number or weight may be employed for diagnosis of  
3 bladder or prostate cancer; analysis of menstrual blood for 8OH2'dG leads  
4 may be employed for early detection of ovarian cancer; analysis of phlegm for  
5 elevated levels of 8OH2'dG may be employed to provide an early test for lung  
6 cancer; and analysis of throat swabs may be employed to provide an early test  
7 for throat or esophageal cancer.

8 While not wishing to be bound by theory, it is believed that 8OH2'dG  
9 free level measurements represent the combined effect of the rate of DNA  
10 damage and repair caused by disease conditions or environmental insults in a  
11 living organism. Essentially, it is believed that the whole body rate of  
12 production of 8OH2'dG is:

- 13 (a) constant in an individual over time in the absence of  
14 disease or environmental insult;
- 15 (b) genetically determined;
- 16 (c) not related to whole body rate of Hydroxyl radical  
17 production;
- 18 (d) increased by environmental insult from putative DNA  
19 adducts (e.g. pyrenes, Toluidine Arsenic, etc.);
- 20 (e) increased in several neurodegenerative diseases;
- 21 (f) assessed equally well by spot and 24 hour urines;
- 22 (g) not affected by antioxidants tocopherol and ascorbate;
- 23 and
- 24 (h) not affected by diet and converted (via HCl and  
25 glycolase) to 8OH2'dGua in the stomach.

26 The clearance rate from cells and the body as a whole is believed to be  
27 very rapid. Normally clearance is via urine but also may be by saliva and  
28 sweat. If rates are accounted for there are compartmental correlations among

1 plasma and urine and saliva and DNA damage levels in leucocytes.

2 Typically, ca. 30% of the whole body 8OH2'dG output is from the brain .

3       Compartmental correlations can be used to determine specific organ  
4 involvement. For example, in *C. Elegans* culture there are bursts of  
5 production during rapid mitosis. Isolated Mitochondrial preparations also  
6 produce 8OH2'dG in respiration. The relationships among different adducts  
7 are different in rapid mitosis, stable cell number cultures and mitochondrial  
8 cultures. Levels in DNA are only weakly correlated with rate of production.

9       Physiologically or transport isolated reservoirs concentrate the  
10 products and magnify the effects of DNA damage and repair, e.g. although I  
11 have measured only an 11% increase in urine levels of smokers, I have  
12 measured a 400% increase in pharangeal swab samples.

13       Thus, it is believed that rates of DNA damage are primarily affected  
14 by: (a) factors causing changes in the conformation of the DNA (mitosis,  
15 binding of endogenous and exogenous adducts, transcription); and (b)  
16 increases in free radical production in subcellular structures in close  
17 dimensional proximity to the DNA (lipid bilayer, nuclear cytosol). The  
18 damage and excision product profiles differ for mitochondrial and nuclear  
19 DNA and by mechanism of damage. These observations are for cervical  
20 cancer, but they are believed equally generalized to lung, lower bowel/colon,  
21 prostate, bladder cancer, etc. as follows:

22       1.       The cervix and the extracellular matrix of the cervix is an in situ  
23 semi-isolated physiological area with respect to transport of excreted cellular  
24 metabolites.

25       2.       The product 8OH2'dG of the combined process of DNA damage  
26 and repair is concentrated in the extracellular matrix of the cervix and  
27 magnify and reflect the effect of these processes more than the levels of the  
28 damage products remaining in the DNA.

- 1           3.       Correlative or normalizing markers for control of sample  
2     dilution and acquisition effects can be selected from metabolites that are (a)  
3     not affected by free radical processes, (b) excreted from the cells at a rapid  
4     rate, or (c) unique or predominant to the metabolism of cervical cells.
- 5           4.       The rate of production of DNA damage/repair products will be  
6     increased when the cells in the cervix are (a) undergoing rapid division, (b)  
7     exposed locally to increased free radical insult as from infection or  
8     inflammation, (c) exposed to increased levels of DNA conformation  
9     modifying adducts from endogenous processes (metabolic shifts) or  
10    exogenous sources (bacterial, or viral infections and/or environmental  
11    toxins).
- 12          5.       The combination of the factors increasing the rate of DNA  
13    damage will constitute a risk factor for cancer which will be reflected in  
14    higher levels of DNA damage and repair product 8OH2'dG in the  
15    extracellular matrix.
- 16          6.       The magnitude of the different mechanisms of DNA damage  
17    will differ and rapid cell division will predominate. Thus, rates of DNA  
18    damage in cancer cases and extracellular levels of 8OH2'dG will be  
19    significantly higher than in cases of ASCUS or with dysplasia or other risk  
20    factors.
- 21          7.       The relative levels of DNA damage and repair products will  
22    differ for different mechanisms. Cancer cells will excrete a significant portion  
23    of the endonuclease excised 8OH2'dG as the phosphate or monophosphate,  
24    and excise a higher proportion of the damaged base by glycolase excision  
25    resulting in elevated levels of 8OH2'dGua in the extracellular matrix. Cancer  
26    cells will also preferentially excrete elevated levels of 5-Oxocytidine and  
27    reduced levels of 8-nitro guanine.
- 28          8.       The absolute and relative levels of 8OH2'dG, 8OH2'dG  
29    Phosphates, 8OH2'dGua, 8 nitroguanine and 5 Oxocytidine will constitute a

1 profile indicating the presence of cancer independent of the cytological results  
2 of the PAP smear and levels of risk factors.

3 9. The absolute and relative levels of DNA damage products will  
4 provide an early indication of cells that are in a precancerous state.

5 10. Co-determined markers of other free radical damage products  
6 will serve to further differentiate the nature of and extent of damage from  
7 other risk factors to the rate of DNA damage. Notably chlorotyrosine will  
8 serve as an indicator of the effect of inflammation or infection and  
9 nitrotyrosine as a normalizer for peroxynitrite DNA damage processes.

10 It is thus seen that:

11 1. The levels of 8OH2'dG in cervical vaginal lavage normalized by  
12 protein or in PAP smear samples from controls normalized by weight are ca  
13 250-500 pg/ml of extracellular matrix or ca 25-50 times plasma. This is in  
14 contrast to the free extracellular levels in muscle, brain, liver, etc. which are on  
15 the order of ca 1-3pg/ml. Therefore, the cervix is seen as semi-isolated with  
16 respect to metabolite transport. In cancer cases where the levels of 8OH2'dG  
17 in the extracellular matrix are ca 100 times higher than in controls the levels in  
18 DNA from the exfoliated cells are only increased by 35% and the difference is  
19 not statistically significant. So the concentration of the products in the semi-  
20 isolated area magnifies the effect of the DNA damage rate.

21 2. Using pattern generating techniques to compare cell extracts  
22 and extracellular matrix from CVL, PAP smear, buccal cell and alveolar  
23 lavage samples and also plasma, red blood cells and urine, the following has  
24 been observed:

25 In CVL and PAP smear samples 23 candidate peaks were over 10x  
26 more concentrated in the extracellular matrix, 3 where highly elevated or  
27 unique in the CVL/PAP smear samples over all other types. Tyramine was  
28 identified as one of these which should be minimally affected by free radical  
29 processes and was selected as a sample acquisition normalizer. Note on an

1 operational basis these studies also served to provide acceptable levels of  
2 sample quality indicators, e.g. uric acid for urine contamination and  
3 glutathione for blood inclusion.

4 3. The ratio of 8OH2'dG/tyramine seems to indicate that the rate  
5 of damage is higher with cancer and with various risk factors which may  
6 include HPV infection, other STD's, multiple partners, hisuitism, endocrine  
7 abnormalities, and various equivocal findings from colposcopy.

8 4. There appears to be a gradation in the increase in rate of DNA  
9 damage that is generally in the same direction as increase in risk factors.

10 5. Even if any of the assumptions are wrong, the rates of local  
11 DNA damage in the cervix in the cancer cohort is much higher than in the  
12 controls or at risk cohort. Thus, a viable cancer screening test subsists.

13 6. Some 8OH2'dG phosphate is always seen in cancer. Thus, when  
14 it is seen in subjects with high rates of DNA damage and other risk factors it is  
15 likely that they actually do have cancer, but it just hasn't advanced to a point  
16 where it can be conventionally detected yet. The 8OH2'dGua/8OH2'dG ratio  
17 was 20 fold higher in two cancer patients than in 8 controls and an algorithm  
18 based on ratio and absolute level separates the categories by a factor of 20,000.

19 The invention is susceptible to modification. For example, the above  
20 technique may be employed to assay free levels of the primary oxidative  
21 DNA excision product 8-hydroxy-2'-deoxyguanosine (8OH2'dG) and sample  
22 normalizing compounds in pharyngeal swabs, induced sputum, bronchial  
23 and alveolar lavage using carbon column switching liquid chromatography  
24 electrochemical detection (LCEC) as above described. Preliminary work  
25 shows three fold elevations of 8OH2'dG in sputum and pharyngeal swabs in  
26 smokers. The absolute levels of 8OH2'dG are 10-30 times greater than in  
27 plasma which supports an initial hypothesis that the respiratory tract is a  
28 semi-isolated physiological transport limited area in which the process of  
29 DNA damage and repair will be magnified. In the cervix, a similarly semi-

1 isolated physiological area, cancer patients shown 2000-10000 fold elevations  
2 of 8OH2'dG over controls. Thus, there is provided a diagnostic or risk  
3 assessment biochemical test for respiratory lesions.

4 As before, and while not wishing to be bound by theory, the overall  
5 hypotheses is that:

6 1. The respiratory tract is a semi-isolated physiologically transport  
7 limited area in which the effects of DNA lesion repair will be magnified.

8 2. Increased DNA damage will be coupled with up regulated  
9 repair and thus the free levels of repair products in the extracellular matrix of  
10 the respiratory tract and thus will be more indicative of risk or presence of  
11 disease than the levels in the DNA itself.

12 3. The levels and nature of DNA and RS damage products will  
13 differ among processes of DNA ligand formation, increased reactive species  
14 product, inflammation/infection and rapid cell mitosis, necrosis and  
15 apoptosis and thus provide a route to diagnosis or risk assessment.

16 The assay of 8OH2'dG and potential sample normalizers in pharyngeal  
17 swabs, induced sputum and bronchial alveolar lavage samples, urine, plasma  
18 and leukocytes from cohorts of controls, individuals with various risk factors  
19 and individuals with respiratory tract lesions using existing technologies  
20 permits the determination of the correlations and compartmental  
21 relationships among the different sample types and the different diagnostic  
22 categories for 8OH2'dG and sample normalizers. This in turn permits  
23 evaluation of correlative and process relevant reactive species markers: 8-  
24 Hydroxyguanine, 8-Hydroxyguanosine (glycolase excision and RNA damage  
25 markers); 7-Methyl guanine (DNA methylation marker); 3-Nitrotyrosine and  
26 8-Nitroguanine (peripheral and DNA reactive nitrogen markers) and 3-  
27 Chlorotyrosine (infection or inflammation marker).

28 The invention also provides strong evidence for concurrent  
29 methylation increases in N methyl and NNdimethyl Serotonin (NM5HT

1 NNM5HT) and 7Nmethyl Guanine (7MG) in plasma and CSF and reduction  
2 in the oxidative DNA damage marker 8-Hydroxy 2'Deoxyguanosine  
3 (8OH2'dG) in stroke and ischemia in humans. More particularly employing  
4 the analysis techniques of the present invention shows a reduction in CNS  
5 production of the oxidative DNA damage product 8-Hydroxy-2'-  
6 deoxyguanosine (8OH2'dG) and an increase in the production of methylated  
7 compounds in a cohort of stroke patients and a similar reduction of 8OH2'dG  
8 levels in animal stroke models. Thus, elucidation of the biochemical  
9 mechanisms of methylation and oxidative free radical damage to DNA and  
10 other cellular structures in stroke and related disorders, and the  
11 determination of the relationships of the biochemistry to stroke management,  
12 provides a rapid single assay technology for methylated, reactive oxygen and  
13 nitrogen species (ROS, RNS) markers in clinically accessible samples and  
14 samples relevant to mechanistic studies in animal models. This provides for  
15 clinical management of and the evaluation of pharmacological intervention in  
16 stroke.

17 Preliminary work with purine specific carbon column switching  
18 techniques as above described also demonstrates elevations of the DNA  
19 hydroxy radical damage marker 8-hydroxy-2'-deoxyguanosine in a number of  
20 neurodegenerative diseases (NDD). Progressive increases with disease have  
21 been shown in, e.g. amyotrophic lateral sclerosis. That is to say, preliminary  
22 work with carbon column switching LCEC technology in accordance with the  
23 present invention has shown that the ROS DNA damage marker 8-hydroxy-  
24 2'deoxyguanosine (8OH2'dG) is elevated in Parkinson's Disease (PD) (urine,  
25 plasma, and CSF), Alzheimer's Disease (AD) (plasma and urine, Huntington's  
26 Disease (HD) (urine), Freidrich's Ataxia (FA) (urine plasma) and that it is  
27 elevated and progressive with time in Amyotrophic Lateral Sclerosis (ALS)  
28 (urine plasma and CSF) but not in myopathies. Secondary evidence from the  
29 patterns of chromatograms determining 8OH2'dG indicate differences in



- 1 unidentified purine adducts in urine plasma and CSF among the  
2 neurodegenerative disorder (NDD) categories. Again, while not wishing to  
3 be bound by theory, the overall hypothesis is as follows:
- 4 1. DNA damage products resulting from processes of  
5 methylation/ROS/RNS/RCLS are different among controls and NDD.
  - 6 2. Ligand of endogenous/exogenous compounds with DNA play a  
7 role in increased DNA damage.
  - 8 3. Excision and repair processes differ among controls and NDD.
  - 9 4. The sites of attack of RS on DNA differ among controls and  
10 NDD.

11 As mentioned supra, other DNA and RNA damage markers and free  
12 radical markers may be assayed as indicators for the presence of or risk of  
13 various health disorders.

14 Table IV below lists preliminary conditions developed for DNA  
15 damage markers and free radical markers established for the purpose of  
16 establishing approximate levels of the analytes of interest. Urine, plasma and  
17 cervical extracellular matrix values are presented for pooled control samples.  
18 The basic sensitivity of all procedures is from 10-50 pg/ml (500-2500 fg on  
19 column).

20

TABLE IV

					pg/mL	ug/gm creatinine	pg/gm
	A	B/D	C1/C2	G1/G2	Plasma	Urine	Cervical
8OH2'dG					10.3	3.86	200
O6MG	20%MEOH(B1)	6%An(B2)ADN	1/1	A/B	0.5	0.32	44
2'dG/7MG	10%MEOH3%An(B1)	7%An(B2)ADN	2/1	A/B	500/3	ND/0.6	2000/15
8OHG	1%MEOH(B1)	1%An(B2)ADN	1/1	B/B	26	9.83	80
8NG	25%MEOH4%AN(B2)	18%An(B1)NBA	1/1	A/B	ND	ND	(?)
8OH2'dA	8%MEOH(B1)	4%An(B2) DD	2/1	A/A	ND	0.60	30
5OH2'dC y	1%MEOH(B3)	1%An(B2)ADN	1/1	B/C	ND	ND	30
5OHU	1%MEOH(B3)	1%An(B3)ADN	1/1	B/C	ND	0.40	56
3NT/3CIT	10%MEOH3%AN(B1)	7%An(B1)NBA	2/1	B/B	3.7/1.1	ND	20/10

MEOH - methanol An-acetonitrile; B1 - lithium acetate 0.1M pH 6; B2 - lithium phosphate 0.1M pH 3; B3 - pentone sulfonic acid 0.1M pH 4; Column Types: 1- Tosobaas C18 ODS 80 TM; 2 - YMC C8 Y02H1 carbon column; A- 4.6 mm d x 4.6 mm; B - 4.6 mm d x 8 mm; C - 4.6 mm d x 13 mm; ADN - adenosine; DD - dodecane; NBA - nitrobenzoic acid; ND - not detected

It is thus seen that the present invention provides an objective reproducible test for cervical cancer and pre-cancer screening, as well as for early detection of various other cancers and other health conditions including heart disease and various degenerative diseases.

CLAIMS

- 1  
2           1.       A method for detecting a health condition of a living organism  
3 comprising electrochemically analyzing samples from selected areas of the  
4 body of said living organism for elevated free levels of nucleotide excision  
5 products resulting from DNA or RNA damage.
- 6           2.       A method according to claim 1, wherein said nucleotide excision  
7 products are selected from the group consisting of 8OH2'dG, O6MG, 2'dG,  
8 7MG, 8NG, 8OHG, 8OH2'dA, 8OHA, 5)H2'dcy, 5OHU, 3NT and 3CIT.
- 9           3.       A method according to claim 1, wherein the samples comprise  
10 stool samples, and the health condition comprises colon cancer.
- 11          4.       A method according to claim 1, wherein the samples comprise  
12 saliva, and the health condition comprises esophageal or throat cancer.
- 13          5.       A method according to claim 1, wherein the samples comprise  
14 urine, and the health condition comprises bladder or testicular cancer.
- 15          6.       A method according to claim 1, wherein the samples comprise  
16 phlegm, and the health condition comprises lung cancer.
- 17          7.       A method according to claim 1, wherein the samples comprise  
18 cervico vaginal lavage, an endocervical swab or brush sample, and the health  
19 condition comprises cervical cancer.
- 20          8.       A method according to claim 7, and including the step of  
21 analyzing for Tyramine, and comparing the mole ratios of 8OH2'dG to  
22 Tyramine.
- 23          9.       A method according to claim 1, wherein the samples comprise  
24 buccal or pharyngeal swab samples and the health condition comprises  
25 pharyngeal or nasal cancer.
- 26          10.      A method according to claim 1, wherein the sample comprises  
27 exfoliated urithelial cells and the health condition comprises bladder,  
28 testicular or prostate cancer.

1           11.     A method according to claim 1, wherein the sample comprises  
2 induced sputum, or alveolar or bronchial lavage, and the health condition  
3 comprises lung or bronchial cancer.

4           12.     A method according to claim 1, wherein the sample comprises a  
5 standard PAP smear endocervical swab or brush sample and the health  
6 condition comprises cervical cancer.

7           13.     A method for determining normalizing compounds for sample  
8 volume and acquisition variables for the measurement of nucleotide excision  
9 products resulting from DNA or RNA damage in a living organism  
10 comprising the steps of: determining the levels of potential normalizing  
11 compounds in cellular and extracellular material from a sample site; selecting  
12 compounds that are unique to or enhanced by metabolic processes of that  
13 sample site, selecting from these compounds that are significantly higher in  
14 the extracellular than in the cellular material from that sample site.

15           14.     A method according to claim 13, for detecting esophageal or  
16 throat cancer, wherein the extracellular material comprises saliva, and  
17 wherein the normalizing compound is 4 hydroxy phenyl lactic acid.

18           15.     A method according to claim 13, for detecting lung or bronchial  
19 cancer, wherein the extracellular material comprises sputum, or alveolar or  
20 bronchial lavage, and wherein the normalizing compound is 4-hydroxy  
21 phenyl lactic acid.

22           16.     A method according to claim 13, for detecting cervical cancer;  
23 wherein the extracellular material comprises a cervico vaginal lavage, an  
24 endocervical swab or brush sample, and wherein the normalizing compound  
25 is tyramine and the diagnostic is the ratio of 8OH2'dG to Tyramine.

26           17.     A method according to claim 13, wheein said nucleotide  
27 excision products are selected from the group consisting of 8OH2'dG, O6MG,  
28 2'dG, 7MC, 8NG, 8OHG, 8OH2'dA, 8OHA, 5)H2'dcy, 5OHU, 3NT and 3CIT.

- 1           18.    A method of codetermining chlorotyrosine as a marker of
- 2   infection to improve the diagnostic specificity of the 8OH1'dG and 8OH2'dG
- 3   phosphate levels.
- 4

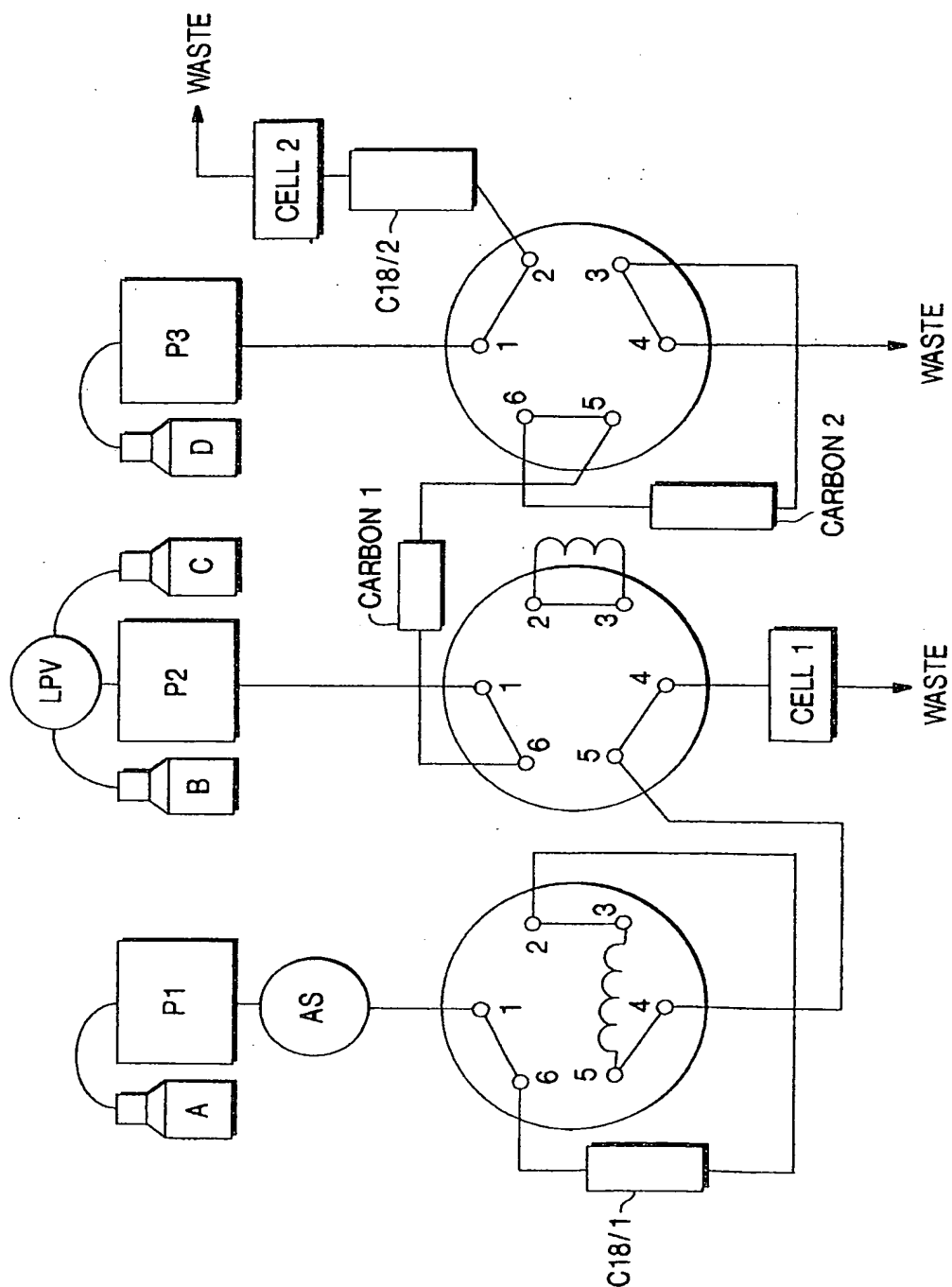
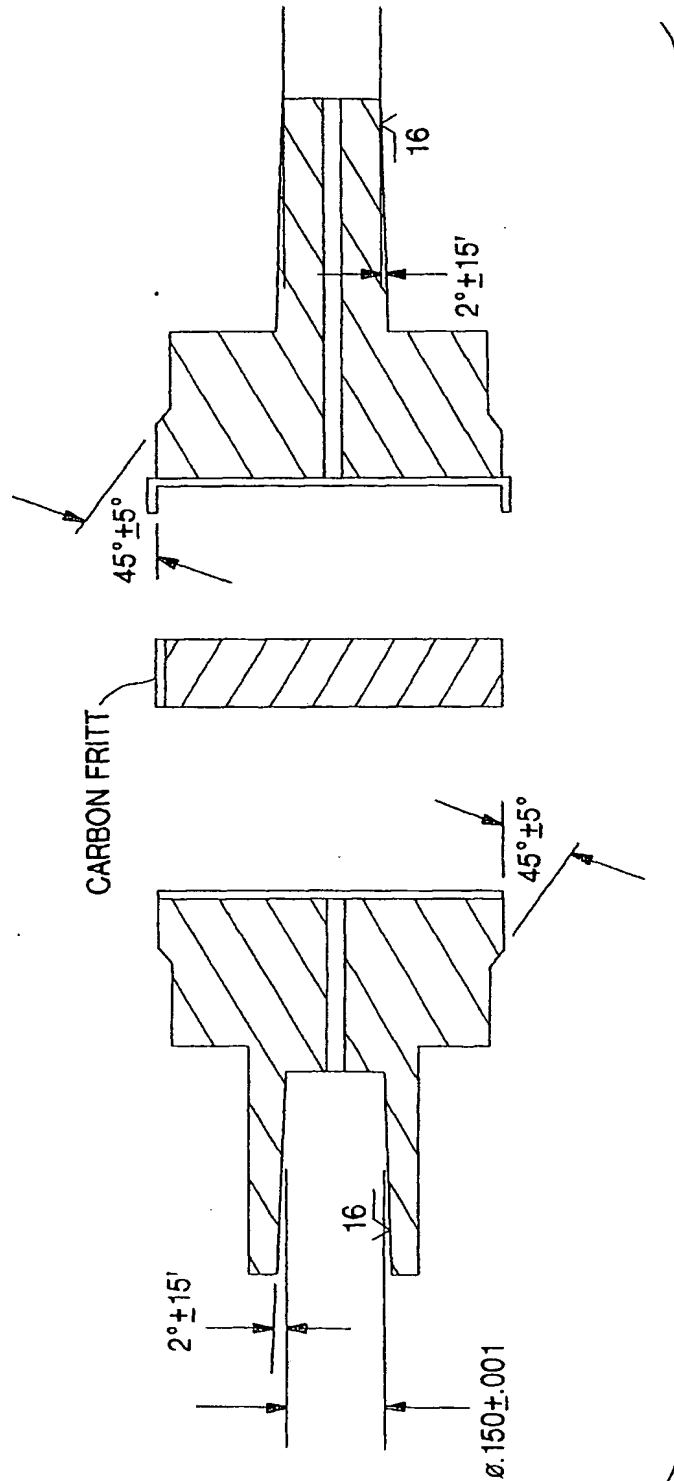
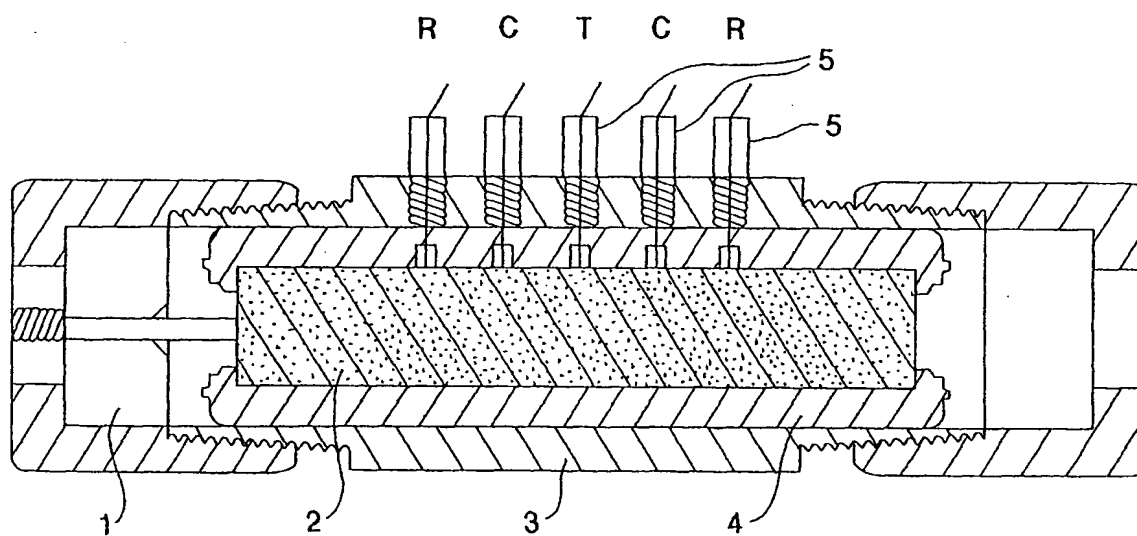


FIG. 1

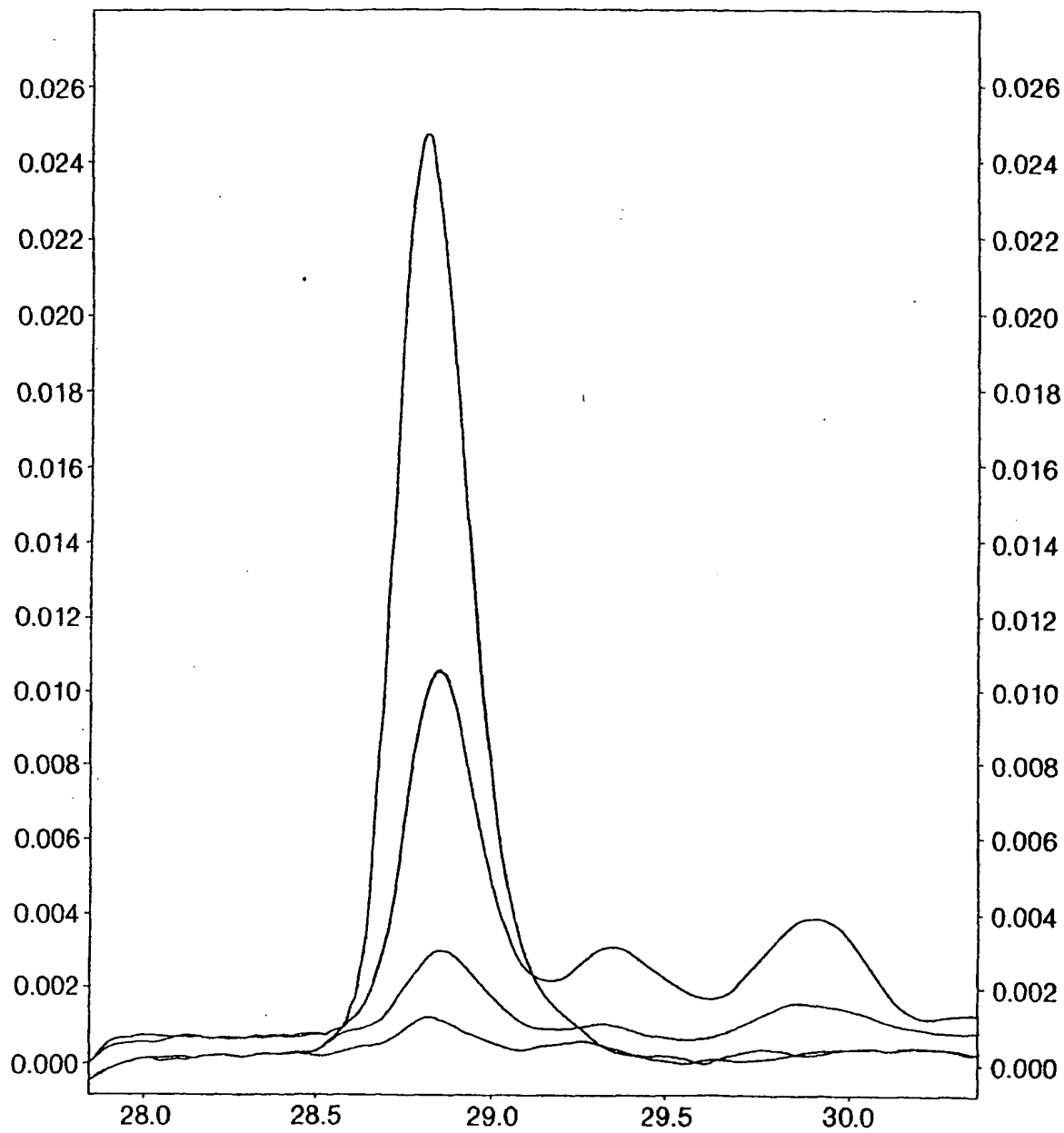
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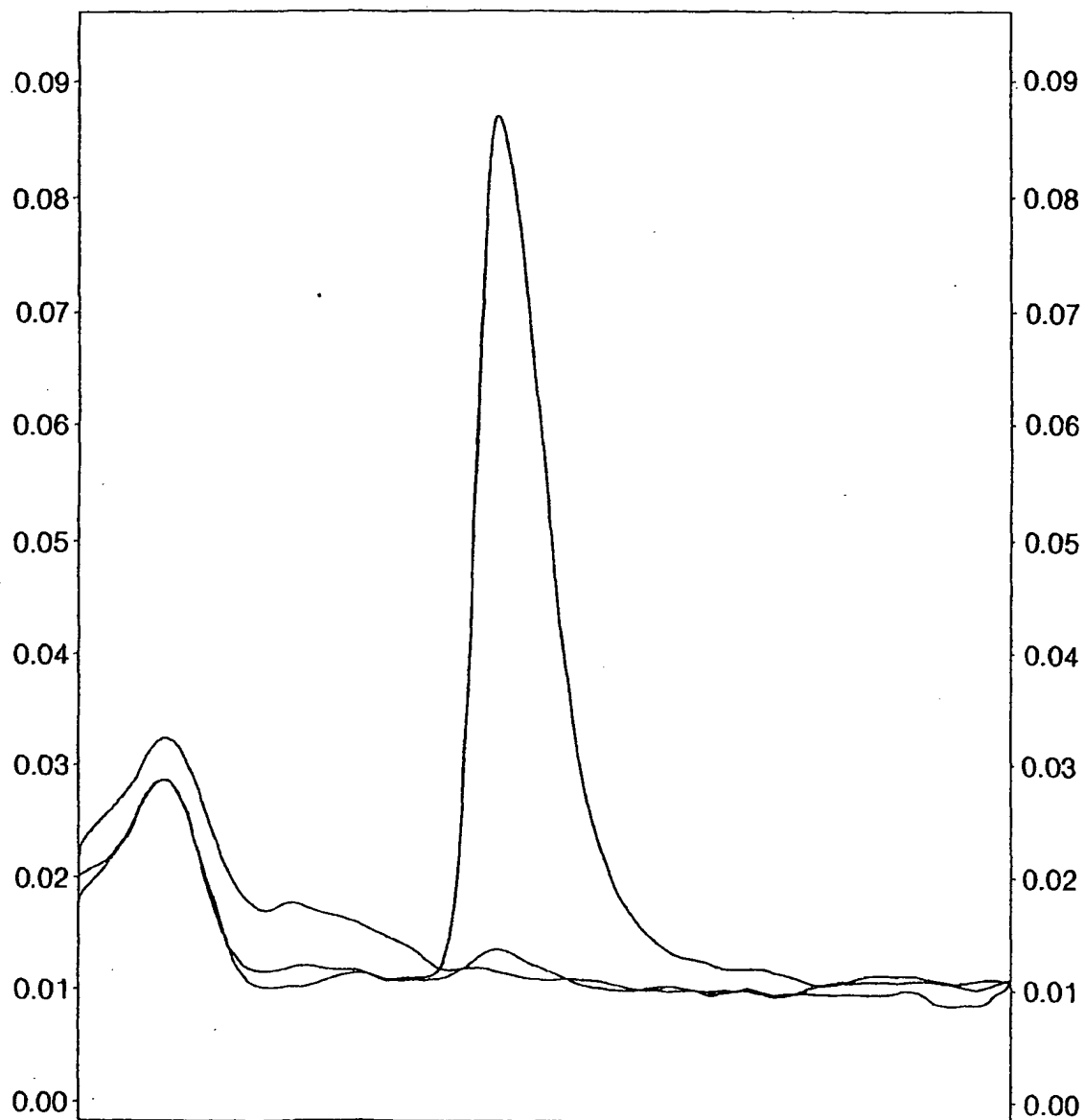
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8OH2'dG levels in four typical normal PAP smear samples  
(top is 200 pg/ml standard)

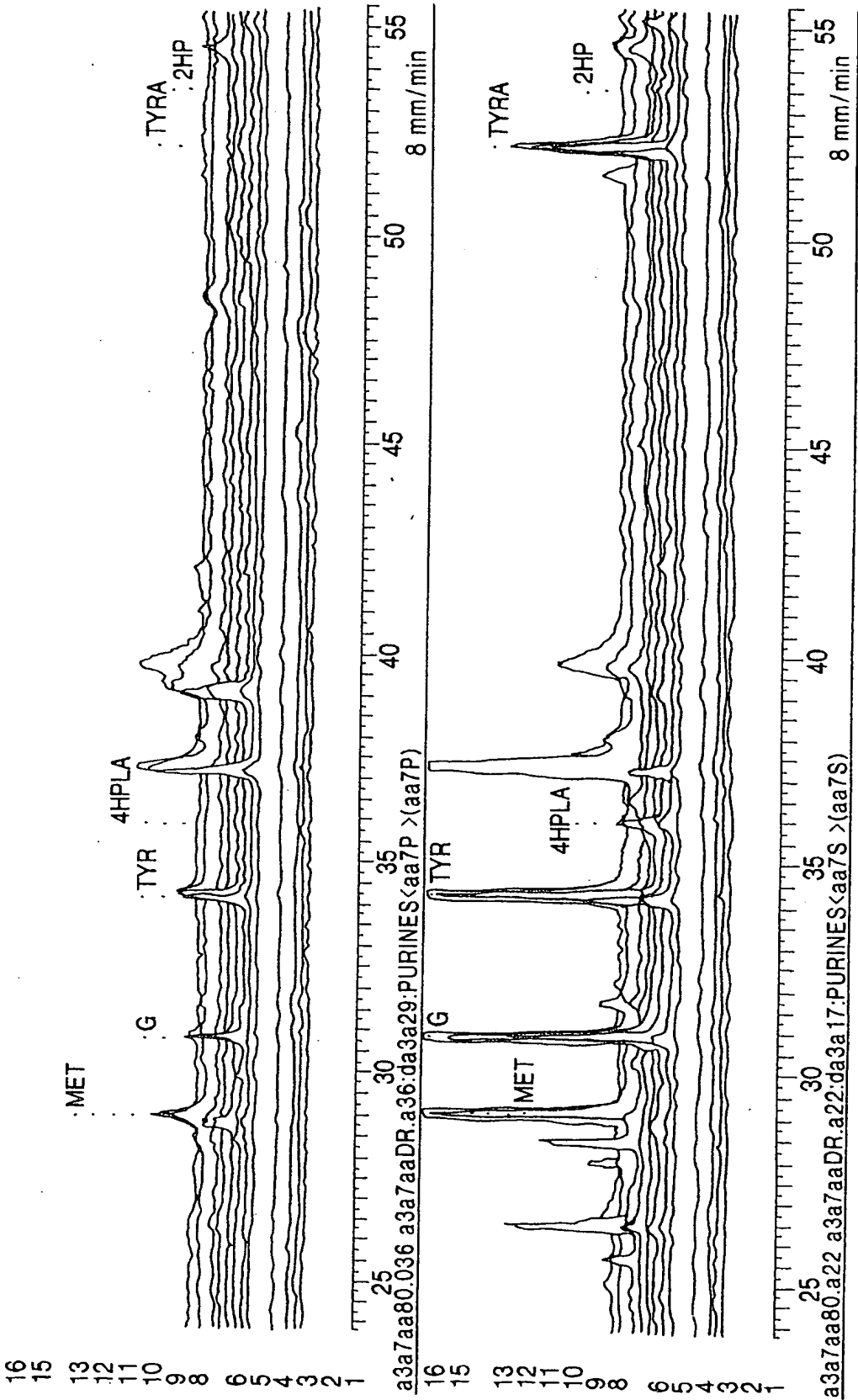
FIG. 4

5/6



O6MG levels in two typical normal PAP smear samples  
(top is 500 pg/ml standard)

**FIG. 5**



Comparison of supernate and cell patterns from PAP smear sample.  
Note: This illustrates one of the criteria for initially selecting tyramine (TYRA) as a normalizer.

FIG. 6